

EPG - DG 1

19. 08. 2005

CLAIMS

1. A method of screening recombinant host cells comprising a ~~gene~~^{gene} library for a protein secreting host cell, the method comprising screening for activity of a secretion stress inducible promoter.
2. The method according to claim 1 comprising the steps of:
 - (i) providing a recombinant host cell comprising the secretion stress inducible promoter operably linked to a nucleic acid sequence encoding a reporter protein or a regulator protein;
 - (ii) introducing the gene library into the host cell of (i);
 - (iii) culturing the host cells obtained in (ii) under conditions promoting expression of the gene library; and
 - (iv) selecting a host cell which expresses the reporter protein or regulator protein.
3. The method according to claim 2, wherein the regulator protein controls the expression of the reporter gene.
4. The method according to claim 3, wherein the regulator protein is an activator or repressor of the expression of the reporter protein.
5. The method according to claim 1, where the host cell is selected from bacterial cells.
6. The method according to claim 5, where the host cells belong to a strain selected from the group consisting of the species *Bacillus alkalophilus*, *Bacillus agaradhaerens*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus clausii*, *Bacillus circulans*, *Bacillus coagulans*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Streptomyces lividans*, *Streptomyces murinus*, *Escherichia coli*, *Lactococcus lactis*, and *Pseudomonas putida*.
7. The method according to claim 1, where the secretion stress inducible promoter is comprised by the nucleic acids 1-999 of SEQ ID NO.:1.
8. The method according to claim 1, where the secretion stress inducible promoter comprises the nucleic acids 1-999 of SEQ ID NO.:1.

9. The method according to claim 1, where the secretion stress inducible promoter consists of the nucleic acids 1-999 of SEQ ID NO.:1.

10. The method according to claim 1, where the secretion stress inducible promoter in its normal position regulates a gene encoded protein that is a functional homolog of the gene encoded protein regulated by the promoter sequence comprised by nucleic acids 1-999 of SEQ ID NO.:1.

11. The method according to claim 1, where the secretion stress inducible promoter in its normal position is the promoter linked to a gene encoding a polypeptide which has at least 70% identity to the amino acid sequence of SEQ ID NO.:2.

12. The method according to claim 1, where the secretion stress inducible promoter is the promoter linked to a gene encoding a polypeptide which has at least 80% identity to the amino acid sequence of SEQ ID NO.:2, or at least 90% identity to the amino acid sequence of SEQ ID NO.:2, or at least 95% identity to the amino acid sequence of SEQ ID NO.:2, or at least 98% identity to the amino acid sequence of SEQ ID NO.:2.

13. The method according to claim 1, where the secretion stress inducible promoter is comprised by the repeated octameric motif of SEQ ID NO.: 3.

14. The method according to claim 1, where the secretion stress inducible promoter comprises the repeated octameric motif of SEQ ID NO.: 3.

15. The method according to claim 1, where the secretion stress inducible promoter is identical to the octameric motif of SEQ ID NO.: 3.

16. The method according to claim 2, where the reporter protein is 2-fold over expressed in a secretion stressed cell compared to a non secretion stressed cell, preferably 5-fold over expressed in a secretion stressed cell compared to a non secretion stressed cell, more preferably 10-fold over expressed in a secretion stressed cell compared to a non secretion stressed cell, or 20-fold over expressed in a secretion stressed cell compared to a non secretion stressed cell, most preferably 50-fold over expressed in a secretion stressed cell compared to a non secretion stressed cell, or more than 100-fold over expressed in a secretion stressed cell compared to a non secretion stressed cell.

17. The method according to claim 2, where the reporter protein is selected from the group consisting of fluorescent protein, antibiotic markers, and substrate converting enzymes.

18. The method according to claim 1, where the secretion stress inducible promoter is comprised by nucleic acids 1-999 of SEQ ID NO.:1, and the host cell further comprises an IPTG-inducible promoter operably linked to a nucleic acid sequence encoding the amino acids 1 to 449 of SEQ ID NO:2.

19. The method according to claim 1, where the secretion stress inducible promoter comprises nucleic acids 1-999 of SEQ ID NO.:1, and the host cell further comprises a IPTG-inducible promoter operably linked to a nucleic acid sequence encoding the amino acids 1 to 449 of SEQ ID NO:2.

20. The method according to claim 1, where the secretion stress inducible promoter consists of nucleic acids 1-999 of SEQ ID NO.:1, and the host cell further comprises a IPTG-inducible promoter operably linked to a nucleic acid sequence encoding the the amino acids 1 to 449 of SEQ ID NO:2.

21. The method according to claim 2, where the protein secreted by the host cell comprises an enzyme.

22. The method according to claim 21, where the enzyme is selected from the group consisting of proteases, cellulases (endoglucanases), beta-glucanases, hemicellulases, lipases, peroxidases, laccases, alfa-amylases, glucoamylases, cutinases, pectinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, pectate lyases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, pectin lyases, mannanases, pectin methylesterases, cello-biohydrolases, transglutaminases and phytases.